PCT/US 98/10951

The Patent Office

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP9 1RH

PRIORITY DOCUMENT

UK 9714316.8

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Crossley

Dated 30 March 1998

P Form 1/77

Pa... Act 1977
(Rule 16)

07 JUL 1997

The Patent Office

1/77

08JUL97 E297587-1 002639
P01/7700 25.00 - 9714316.8

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — 19964PV

2. Patent application numt (The Patent Office will) — 9714316.8 — 07 JUL 1997

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Merck & Co., Inc.
P. O. Box 2000
Rahway, New Jersey 07065-0900
U.S.A.

Patents ADP number (if you know it) — 06767423

If the applicant is a corporate body, give the country/state of its incorporation — New Jersey, USA

4. Title of the invention — Biarylalkanoic acids as cell adhesion inhibitors

5. Name of your agent (if you have one) — Mr. I. J. Hiscock

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Merck & Co., Inc.
European Patent Department
Terlings Park
Eastwick Road
Harlow
Essex CM20 2QR

Patents ADP number (if you know it) — 06546683001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority Application number (if you know it)	Date of filing (day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	0
~~Description~~	37
Claim(s)	7
Abstract	1
Drawing(s)	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	-
Request for preliminary examination and search (Patents Form 9/77)	-
Request for substantive examination (Patents Form 10/77)	-
Any other documents (please specify)	Fee Sheet

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 04 July 1997

Mr. ~~I. J. Hiscock~~

12. Name and daytime telephone number of person to contact in the United Kingdom — Mr. I. J. Hiscock — 01279 440175

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

TITLE OF THE INVENTION
BIARYLALKANOIC ACIDS AS CELL ADHESION INHIBITORS

BACKGROUND OF THE INVENTION

The present invention relates to novel substituted biarylalkanoic acid derivatives which are useful for the inhibition and prevention of leukocyte adhesion and leukocyte adhesion-mediated pathologies. This invention also relates to compositions containing such compounds and methods of treatment using such compounds.

Many physiological processes require that cells come into close contact with other cells and/or extracellular matrix. Such adhesion events may be required for cell activation, migration, proliferation and differentiation. Cell-cell and cell-matrix interactions are mediated through several families of cell adhesion molecules (CAMs) including the selectins, integrins, cadherins and immunoglobulins. CAMs play an essential role in both normal and pathophysiological processes. Therefore, the targetting of specific and relevant CAMs in certain disease conditions without interfering with normal cellular functions is essential for an effective and safe therapeutic agent that inhibits cell-cell and cell-matrix interactions.

The integrin superfamily is made up of structurally and functionally related glycoproteins consisting of α and β heterodimeric, transmembrane receptor molecules found in various combinations on nearly every mammalian cell type. VLA-4 ("very late antigen-4"; CD49d/CD29; or $\alpha 4\beta_1$) is an integrin expressed on all leukocytes, except platelets and mature neutrophils, and is a key mediator of the cell-cell and cell-matrix interactions of leukocytes. The ligands for VLA-4 include vascular cell adhesion molecule-1 (VCAM-1) and the CS-1 domain of fibronectin (FN). VCAM-1 is a member of the Ig superfamily and is expressed *in vivo* on endothelial cells at sites of inflammation and on dendritic and macrophage-like cells. VCAM-1 is produced by vascular endothelial cells in response to pro-inflammatory cytokines. The CS-1 domain is a 25 amino acid sequence that arises by alternative splicing within a region of fibronectin. A role for VLA-

4/CS-1 interactions in inflammatory conditions has been proposed (see M. J. Elices, "The integrin $\alpha_4\beta_1$ (VLA-4) as a therapeutic target." in Cell Adhesion and Human Disease, Ciba Found. Symp., John Wiley & Sons, NY, 1995, p. 79).

Neutralizing anti-α_4 antibodies or blocking peptides that inhibit the interaction between VLA-4 and its ligands have proven efficacious both prophylactically and therapeutically in several animal models of disease, including i) experimental allergic encephalomyelitis, a model of neuronal demyelination resembling multiple sclerosis; ii) bronchial hyperresponsiveness in sheep and guinea pigs as models for the various phases of asthma; iii) adjuvant-induced arthritis in rats as a model of inflammatory arthritis; iv) adoptive autoimmune diabetes in the NOD mouse; v) cardiac allograft survival in mice as a model of organ transplantation; vi) spontaneous chronic colitis in cotton-top tamarins which resembles human ulcerative colitis, a form of inflammatory bowel disease; vii) contact hypersensitivity models as a model for skin allergic reactions; viii) acute neurotoxic nephritis; ix) tumor metastasis; x) experimental autoimmune thyroiditis; and xi) ischemic tissue damage following arterial occlusion in rats. The primary mechanism of action of such antibodies appears to be the inhibition of lymphocyte and monocyte interactions with CAMs associated with components of the extracellular matrix, thereby limiting leukocyte migration to extravascular sites of injury or inflammation and/or limiting the priming and/or activation of leukocytes.

There is additional evidence supporting a possible role for VLA-4 interactions in other diseases, including rheumatoid arthritis; various melanomas, carcinomas, and sarcomas; inflammatory lung disorders; atherosclerotic plaque formation; restenosis; and circulatory shock.

At present, there is only a humanized monoclonal antibody (Antegren®) against VLA-4 in clinical development for the treatment of "flares" associated with multiple sclerosis. Several peptidyl antagonists of VLA-4 have been described (US 5,510,332, WO97/03094, WO97/02289, WO96/40781, WO96/22966,

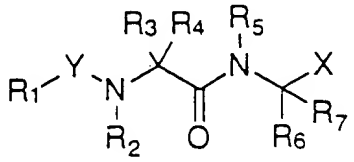
WO96/20216, WO96/01644, WO96/06108, WO95/15973). There is one report of nonpeptidyl inhibitors of the ligands for α_4-integrins (WO96/31206). There still remains a need for low molecular weight, specific inhibitors of VLA-4-dependent cell adhesion that have improved pharmacokinetic and pharmacodynamic properties such as oral bioavailability and significant duration of action. Such compounds would prove to be useful for the treatment, prevention or suppression of various pathologies mediated by VLA-4 binding and cell adhesion and activation.

SUMMARY OF THE INVENTION

The compounds of the present invention are antagonists of the VLA-4 integrin ("very late antigen-4"; CD49d/CD29; or $\alpha_4\beta_1$), thereby blocking the binding of VLA-4 to its various ligands, such as VCAM-1 and regions of fibronectin. Thus, these antagonists are useful in inhibiting cell adhesion processes including cell activation, migration, proliferation and differentiation. These antagonists are useful in the treatment, prevention and suppression of diseases mediated by VLA-4 binding and cell adhesion and activation, such as multiple sclerosis, asthma, allergic rhinitis, allergic conjunctivitis, inflammatory lung diseases, rheumatoid arthritis, septic arthritis, type I diabetes, organ transplantation, restenosis, autologous bone marrow transplantation, inflammatory sequelae of viral infections, myocarditis, inflammatory bowel disease including ulcerative colitis and Crohn's disease, certain types of toxic and immune-based nephritis, contact dermal hypersensitivity, psoriasis, tumor metastasis, and atherosclerosis.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel compounds of Formula I



I

or a pharmaceutically acceptable salt thereof wherein:

R^1 is 1) C_{1-10}alkyl,
2) C_{2-10}alkenyl,
3) C_{2-10}alkynyl,
4) Cy,
5) Cy-C_{1-10}alkyl,
6) Cy-C_{2-10}alkenyl,
7) Cy-C_{2-10}alkynyl,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a; and Cy is optionally substituted with one to four substituents independently selected from R^b;

R^2 and R^3 are independently
1) hydrogen, or
2) a group selected from R^1; or

R^2 and R^3 together with the atoms to which they are attached form a ring of 4 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein said ring may be isolated or benzo-fused, and optionally substituted with one to four substituents independently selected from R^b;

R^4 and R^7 are independently selected from the group consisting of
1) hydrogen,
2) C_{1-10}alkyl,
3) C_{2-10}alkenyl,
4) C_{2-10}alkynyl,
5) aryl,
6) aryl C_{1-10}alkyl,
7) heteroaryl, and
8) heteroaryl C_{1-10}alkyl,

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents independently selected from R^a, and aryl and heteroaryl are optionally substituted with one to four substituents independently selected from R^b; or

R3, R4 and the carbon to which they are attached form a 3-7 membered ring optionally containing 0-2 heteroatoms selected from N, O and S;

R^5 is 1) hydrogen,
2) C_{1-10}alkyl optionally substituted with one to four substituents independently selected from R^a, or
3) Cy optionally substituted with one to four substituents independently selected from R^b,

R^6 is 1) Ar^1-Ar^2-C_{1-10}alkyl,
2) Ar^1-Ar^2-C_{2-10}alkenyl,
3) Ar^1-Ar^2-C_{2-10}alkynyl,
wherein Ar^1 and Ar^2 are independently aryl or heteroaryl each of which is optionally substituted with one to four substituents independently selected from R^b; alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents independently selected from R^a;

R^a is 1) Cy
2) $-OR^d$,
3) $-NO_2$,
4) halogen
5) $-S(O)_mR^d$,
6) $-SR^d$,
7) $-S(O)_2OR^d$,
8) $-S(O)_mNR^dR^e$,
9) $-NR^dR^e$,
10) $-O(CR^fR^g)_nNR^dR^e$,
11) $-C(O)R^d$,

12) $-CO_2R^d$,
13) $-CO_2(CR^fR^g)_nCONR^dR^e$,
14) $-OC(O)R^d$,
15) $-CN$,
16) $-C(O)NR^dR^e$,
17) $-NR^dC(O)R^e$,
18) $-OC(O)NR^dR^e$,
19) $-NR^dC(O)OR^e$,
20) $-NR^dC(O)NR^dR^e$,
21) $-CR^d(N\text{-}OR^e)$, or
22) $-CF_3$;

wherein Cy is optionally subsituted with one to four substituents independently selected from R^c;

R^b is 1) a group selected from R^a,
2) $C_{1\text{-}10}$ alkyl,
3) $C_{2\text{-}10}$ alkenyl,
4) $C_{2\text{-}10}$ alkynyl,
5) aryl $C_{1\text{-}10}$alkyl,
6) heteroaryl $C_{1\text{-}10}$ alkyl,

wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl are optionally substituted with a group independently selected from R^c;

R^c is 1) halogen,
2) amino,
3) carboxy,
4) $C_{1\text{-}4}$alkyl,
5) $C_{1\text{-}4}$alkoxy,
6) aryl,
7) aryl $C_{1\text{-}4}$alkyl, or
8) aryloxy.

R^d and R^e are independently selected from hydrogen, $C_{1\text{-}10}$alkyl,

C_{2-10}alkenyl, C_{2-10}alkynyl, Cy and Cy C_{1-10}alkyl, wherein alkyl, alkenyl, alkynyl and Cy is optionally substituted with one to four substituents independently selected from R^c; or
R^d and R^e together with the atoms to which they are attached form a heterocyclic ring of 5 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen;

R^f and R^g are independently selected from hydrogen, C_{1-10}alkyl, Cy and Cy C_{1-10}alkyl; or
R^f and R^g together with the carbon to which they are attached form a ring of 5 to 7 members containing 0-2 heteroatoms independently selected from oxygen, sulfur and nitrogen;

R^h is 1) hydrogen,
2) C_{1-10}alkyl,
3) C_{2-10}alkenyl,
4) C_{2-10}alkynyl,
5) cyano,
6) aryl,
7) aryl C_{1-10}alkyl,
8) heteroaryl,
9) heteroaryl C_{1-10}alkyl, or
10) $-SO_2R^i$;
wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a; and aryl and heteroaryl are each optionally substituted with one to four substituents independently selected from R^b;

R^i 1) C_{1-10}alkyl,
2) C_{2-10}alkenyl,
3) C_{2-10}alkynyl, or
4) aryl;
wherein alkyl, alkenyl, alkynyl and aryl are each optionally substituted with one to four substituents independently selected from R^c;

Cy is cycloalkyl, heterocyclyl, aryl, or heteroaryl;

m is an integer from 1 to 2;

n is an integer from 1 to 10;

X is 1) $-C(O)OR^d$,
2) $-P(O)(OR^d)(OR^e)$
3) $-P(O)(R^d)(OR^e)$
4) $-S(O)_mOR^d$,
5) $-C(O)NR^dR^h$, or
6) -5-tetrazolyl;

Y is 1) -C(O)-,
2) -O-C(O)-,
3) $-NR^e-C(O)-$,
4) $-S(O)_2-$,
5) $-P(O)(OR^i)$
6) C(O)C(O).

In one embodiment, R^1 is aryl or heteroaryl each optionally substituted with one to three groups selected from R^b. In a preferred embodiment R^1 is phenyl optionally substituted with one to three groups selected from R^b.

In another embodiment, R^2 is hydrogen, C_{1-10} alkyl, Cy or Cy-C_{1-10} alkyl; or R^2, R^3 together with the atoms to which they are attached form a ring of 4 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein said ring may be isolated or benzo-fused, and optionally substituted with one to four substituents independently selected from R^b. Preferably R2, R3 together with the atoms to which they are attached for a ring of 5 to 6 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein said

ring may be isolated or benzo-fused, and optionally substituted with one to four substituents independently selected from R^b.

In another embodiment R^4 is H, C_{1-10}alkyl, aryl, heteroaryl, aryl-C_{1-10}alkyl or heteroaryl-C_{1-10}alkyl. Preferably, R^4 is H or C1-10 alkyl.

In another embodiment R^6 is Ar^1-Ar^2-C_{1-10}alkyl. Preferably R^6 is Ar^1-Ar^2-C_{1-3}alkyl.

In another embodiment X is C(O)OR^d.

In yet another embodiment Y is C(O) or $S(O)_2$.

Representative compounds of formula I are as follows:
N-(3,4-dimethoxybenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline-3(S)-carbonyl-(L)-4-biphenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-biphenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L-prolyl-(L)-4-(4-fluorophenyl)phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-thienyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(3'-thienyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(4'-trifluoromethyl-phenyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-methoxy-phenyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-formyl-phenyl)-phenylalanine.

"Alkyl", as well as other groups having the prefix "alk", such as alkoxy, alkanoyl, means carbon chains which may be linear or branched or combinations thereof. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec- and tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, and the like.

"Alkenyl" means carbon chains which contain at least one carbon-carbon double bond, and which may be linear or branched or combinations thereof. Examples of alkenyl include vinyl, allyl,

isopropenyl, pentenyl, hexenyl, heptenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, and the like.

"Alkynyl" means carbon chains which contain at least one carbon-carbon triple bond, and which may be linear or branched or combinations thereof. Examples of alkynyl include ethynyl, propargyl, 3-methyl-1-pentynyl, 2-heptynyl and the like.

"Cycloalkyl" means mono- or bicyclic saturated carbocyclic rings, each of which having from 3 to 10 carbon atoms. The term also inecludes monocyclic ring fused to an aryl group in which the point of attachment is on the non-aromatic portion. Examples of cycloalkyl include cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, tetrahydronaphthyl, decahydronaphthyl, indanyl, and the like.

"Aryl" means mono- or bicyclic aromatic rings containing only carbon atoms. The term also includes aryl group fused to a monocyclic cycloalkyl or monocyclic hetercyclyl group in which the point of attachment is on the aromatic portion. Examples of aryl include phenyl, naphthyl, indanyl, indenyl, tetrahydronaphthyl, 2,3-dihydrobenzofuranyl, benzopyranyl, 1,4-benzodioxanyl, and the like.

"Heteroaryl" means a mono- or bicyclic aromatic ring containing at least one heteroatom selected from N, O and S, with each ring containing 5 to 6 atoms. Examples of heteroaryl include pyrrolyl, isoxazolyl, isothiazolyl, pyrazolyl, pyridyl, oxazolyl, oxadiazolyl, thiadiazolyl, thiazolyl, imidazolyl, triazolyl, tetrazolyl, furanyl, triazinyl, thienyl, pyrimidyl, pyridazinyl, pyrazinyl, benzoxazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, benzothiophenyl, furo(2,3-b)pyridyl, quinolyl, indolyl, isoquinolyl, and the like.

"Heterocyclyl" means mono- or bicyclic saturated rings containing at least one heteroatom selected from N, S and O, each of said ring having from 3 to 10 atoms. The term also includes monocyclic heterocycle fused to an aryl or heteroaryl group in which the point of attachment is on the non-aromatic portion. Examples of "heterocyclyl" include pyrrolidinyl, piperidinyl, piperazinyl, imidazolidinyl, 2,3-dihydrofuro(2,3-b)pyridyl, benzoxazinyl,

tetrahydrohydroquinolinyl, tetrahydroisoquinolinyl, dihydroindolyl, and the like.

"Halogen" includes fluorine, chlorine, bromine and iodine.

Optical Isomers - Diastereomers - Geometric Isomers

Compounds of Formula I contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. The present invention is meant to comprehend all such isomeric forms of the compounds of Formula I.

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

Salts

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

It will be understood that, as used herein, references to the compounds of Formula I are meant to also include the pharmaceutically acceptable salts.

Utilities

The ability of the compounds of Formula I to antagonize the actions of VLA-4 integrin makes them useful for preventing or reversing the symptoms, disorders or diseases induced by the binding of VLA-4 to its various ligands. Thus, these antagonists will inhibit cell adhesion processes including cell activation, migration, proliferation and differentiation. Accordingly, another aspect of the present invention provides a method for the treatment (including prevention, alleviation, amelioration or suppression) of diseases or disorders or symptoms mediated by VLA-4 binding and cell adhesion and activation, which comprises administering to a mammal an effective amount of a compound of Formula I. Such diseases, disorders, conditions or symptoms are for example (1) multiple sclerosis, (2) asthma, (3) allergic rhinitis, (4) allergic conjunctivitis, (5) inflammatory lung diseases, (6) rheumatoid arthritis, (7) septic arthritis, (8) type I diabetes, (9) organ transplantation rejection, (10) restenosis, (11) autologous bone marrow transplantation, (12) inflammatory sequelae of viral infections, (13) myocarditis, (14) inflammatory bowel disease including ulcerative colitis and Crohn's disease, (15) certain types of toxic and immune-based nephritis, (16) contact dermal hypersensitivity, (17) psoriasis, (18) tumor metastasis, and (19) atherosclerosis.

Dose Ranges

The magnitude of prophylactic or therapeutic dose of a compound of Formula I will, of course, vary with the nature of the severity of the condition to be treated and with the particular compound of Formula I and its route of administration. It will also vary according to the age, weight and response of the individual patient. In general, the daily dose range lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases.

For use where a composition for intravenous administration is employed, a suitable dosage range is from about 0.001 mg to about 25 mg (preferably from 0.01 mg to about 1 mg) of a compound of Formula I per kg of body weight per day and for cytoprotective use from about 0.1 mg to about 100 mg (preferably from about 1 mg to about 100 mg and more preferably from about 1 mg to about 10 mg) of a compound of Formula I per kg of body weight per day.

In the case where an oral composition is employed, a suitable dosage range is, e.g. from about 0.01 mg to about 100 mg of a compound of Formula I per kg of body weight per day, preferably from about 0.1 mg to about 10 mg per kg and for cytoprotective use from 0.1 mg to about 100 mg (preferably from about 1 mg to about 100 mg and more preferably from about 10 mg to about 100 mg) of a compound of Formula I per kg of body weight per day.

For the treatment of diseases of the eye, ophthalmic preparations for ocular administration comprising 0.001-1% by weight solutions or suspensions of the compounds of Formula I in an acceptable ophthalmic formulation may be used.

Pharmaceutical Compositions

Another aspect of the present invention provides pharmaceutical compositions which comprises a compound of Formula I

and a pharmaceutically acceptable carrier. The term "composition", as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of Formula I, additional active ingredient(s), and pharmaceutically acceptable excipients.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like.

The pharmaceutical compositions of the present invention comprise a compound of Formula I as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

For administration by inhalation, the compounds of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulisers. The compounds may also be delivered as powders which may be formulated and the powder composition may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery system for inhalation is a metered dose inhalation (MDI) aerosol, which may be formulated as a suspension or solution of a compound of Formula I in suitable propellants, such as fluorocarbons or hydrocarbons.

Suitable topical formulations of a compound of formula I include transdermal devices, aerosols, creams, ointments, lotions, dusting powders, and the like.

In practical use, the compounds of Formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets, with the solid oral preparations being preferred over the liquid preparations. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, the compounds of Formula I may also be administered by controlled release means and/or delivery devices such as those described in U.S. Patent

Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,630,200 and 4,008,71:

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Desirably, each tablet contains from about 1 mg to about 500 mg of the active ingredient and each cachet or capsule contains from about 1 to about 500 mg of the active ingredient.

The following are examples of representative pharmaceutical dosage forms for the compounds of Formula I:

Injectable Suspension (I.M.)	mg/mL
Compound of Formula I	10
Methylcellulose	5.0
Tween 80	0.5
Benzyl alcohol	9.0
Benzalkonium chloride	1.0

Water for injection to a total volume of 1 mL

Tablet	mg/tablet
Compound of Formula I	25
Microcrystalline Cellulose	415
Povidone	14.0
Pregelatinized Starch	3.5
Magnesium Stearate	2.5
	500

Capsule	mg/capsule
Compound of Formula I	25
Lactose Powder	573.5
Magnesium Stearate	1.5
	600

Aerosol	Per canister
Compound of Formula I	24 mg
Lecithin, NF Liquid Conc.	1.2 mg
Trichlorofluoromethane, NF	4.025 g
Dichlorodifluoromethane, NF	12.15 g

Combination Therapy

Compounds of Formula I may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which compounds of Formula I are useful. Such other drugs may be administered, by a route and in an amount commonly used therefor, contemporaneously or sequentially with a compound of Formula I. When a compound of Formula I is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of Formula I is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a

compound of Formula I. Examples of other active ingredients that may be combined with a compound of Formula I, either administered separately or in the same pharmaceutical compositions, include, but are limited to:
(a) other VLA-4 antagonists such as those described in US 5,510,332, WO97/03094, WO97/02289, WO96/40781, WO96/22966, WO96/20216, WO96/01644, WO96/06108, WO95/15973 and WO96/31206; (b) steroids such as beclomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and hydrocortisone; (c) immunosuppressants such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants; (d) antihistamines (H1-histamine antagonists) such as bromopheniramine, chlorpheniramine, dexchlorpheniramine, triprolidine, clemastine, diphenhydramine, diphenylpyraline, tripelennamine, hydroxyzine, methdilazine, promethazine, trimeprazine, azatadine, cyproheptadine, antazoline, pheniramine pyrilamine, astemizole, terfenadine, loratadine, cetirizine, fexofenadine, descarboethoxyloratadine, and the like; (e) non-steroidal anti-asthmatics such as β2-agonists (terbutaline, metaproterenol, fenoterol, isoetharine, albuterol, bitolterol, and pirbuterol), theophylline, cromolyn sodium, atropine, ipratropium bromide, leukotriene antagonists (zafirlukast, montelukast, pranlukast, iralukast, pobilukast, SKB-106,203), leukotriene biosynthesis inhibitors (zileuton, BAY-1005); (f) non-steroidal antiinflammatory agents (NSAIDs) such as propionic acid derivatives (alminoprofen, benoxaprofen, bucloxic acid, carprofen, fenbufen, fenoprofen, fluprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, miroprofen, naproxen, oxaprozin, pirprofen, pranoprofen, suprofen, tiaprofenic acid, and tioxaprofen), acetic acid derivatives (indomethacin, acemetacin, alclofenac, clidanac, diclofenac, fenclofenac, fenclozic acid, fentiazac, furofenac, ibufenac, isoxepac, oxpinac, sulindac, tiopinac, tolmetin, zidometacin, and zomepirac), fenamic acid derivatives (flufenamic acid, meclofenamic acid, mefenamic acid, niflumic acid and tolfenamic acid), biphenylcarboxylic acid derivatives (diflunisal and flufenisal), oxicams (isoxicam, piroxicam, sudoxicam and tenoxican), salicylates (acetyl

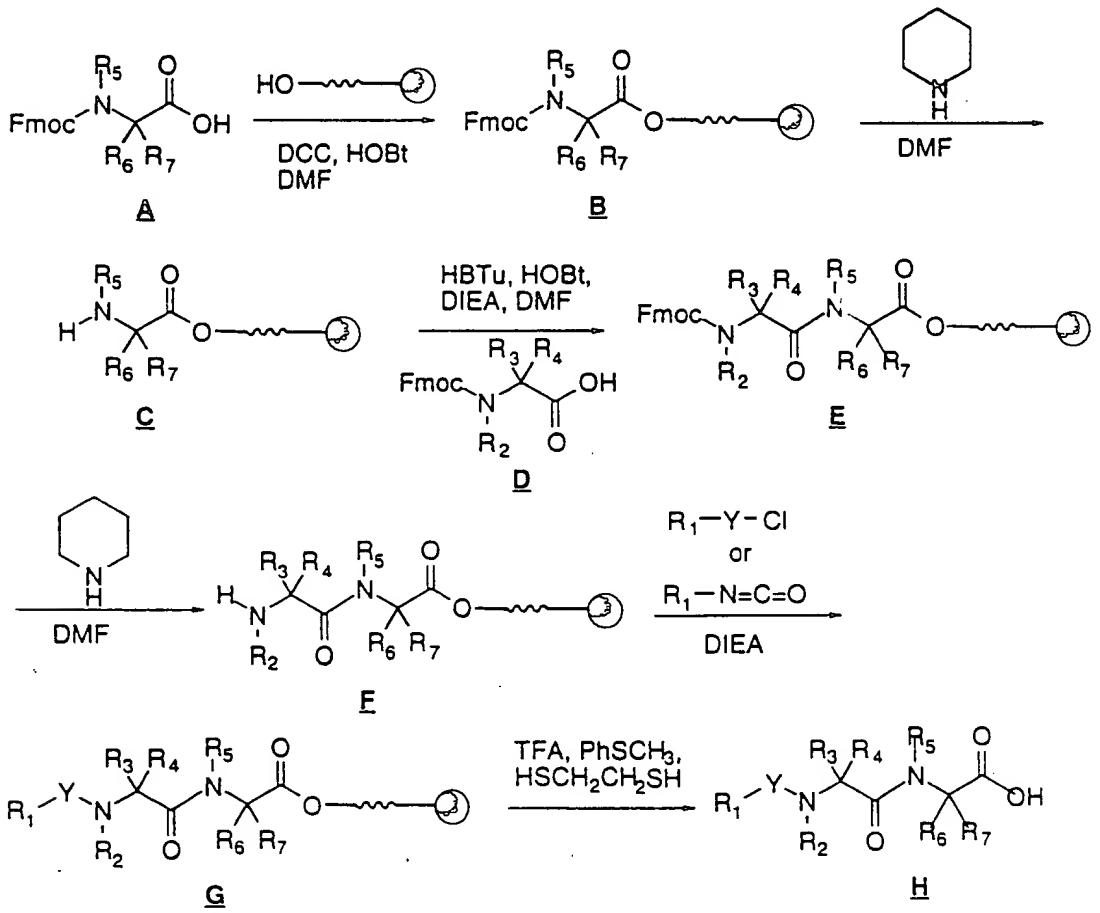
salicylic acid and the phenylbutazones); (g) cyclooxygenase-2 (COX-2) inhibitors; (h) cholesterol lowering agents such as HMG-CoA reductase inhibitors (lovastatin, simvastatin and pravastatin, fluvastatin, atorvastatin, and other statins), sequestrants (cholestyramine and colestipol), nicotinic acid, fenofibric acid derivatives (gemfibrozil, clofibrat, fenofibrate and benzafibrate), and probucol; (i) anti-diabetic agents such as insulin, sulfonylureas, biguanides (metformin), α-glucosidase inhibitors (acarbose) and glitazones (troglitazone and pioglitazone); other compounds such as 5-aminosalicylic acid and prodrugs thereof, antimetabolites such as azathioprine and 6-mercaptopurine, and cytotoxic cancer chemotherapeutic agents.

The weight ratio of the compound of the Formula I to the second active ingredient may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used. Thus, for example, when a compound of the Formula I is combined with an NSAID the weight ratio of the compound of the Formula I to the NSAID will generally range from about 1000:1 to about 1:1000, preferably about 200:1 to about 1:200. Combinations of a compound of the Formula I and other active ingredients will generally also be within the aforementioned range, but in each case, an effective dose of each active ingredient should be used.

Compounds of the present invention may be prepared by procedures illustrated in the accompanying schemes. In the first method (Scheme 1), a resin-based synthetic strategy is outlined where the resin employed is represented by the ball (●). An N-Fmoc-protected amino acid derivative **A** (Fmoc = fluorenylmethoxycarbonyl) is loaded on to the appropriate hydroxyl-containing resin using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF) to give **B**. The Fmoc protecting group is removed with piperidine in DMF to yield free amine **C**. The next Fmoc-protected amino acid derivative **D** is coupled to **C** employing standard peptide (in this instance, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), HOBt, and N,N-diisopropylethylamine (DIEA) in DMF) to yield dipeptide **E**. The

Fmoc group is removed with piperidine in DMF to yield the free amine F. An acid chloride or isocyanate derivative is reacted with F in the presence of DIEA to yield G. The final product is removed from the resin with strong acid (in this instance, trifluoroacetic acid (TFA) in the presence of thioanisole and ethanedithiol) to yield compounds of the present invention H.

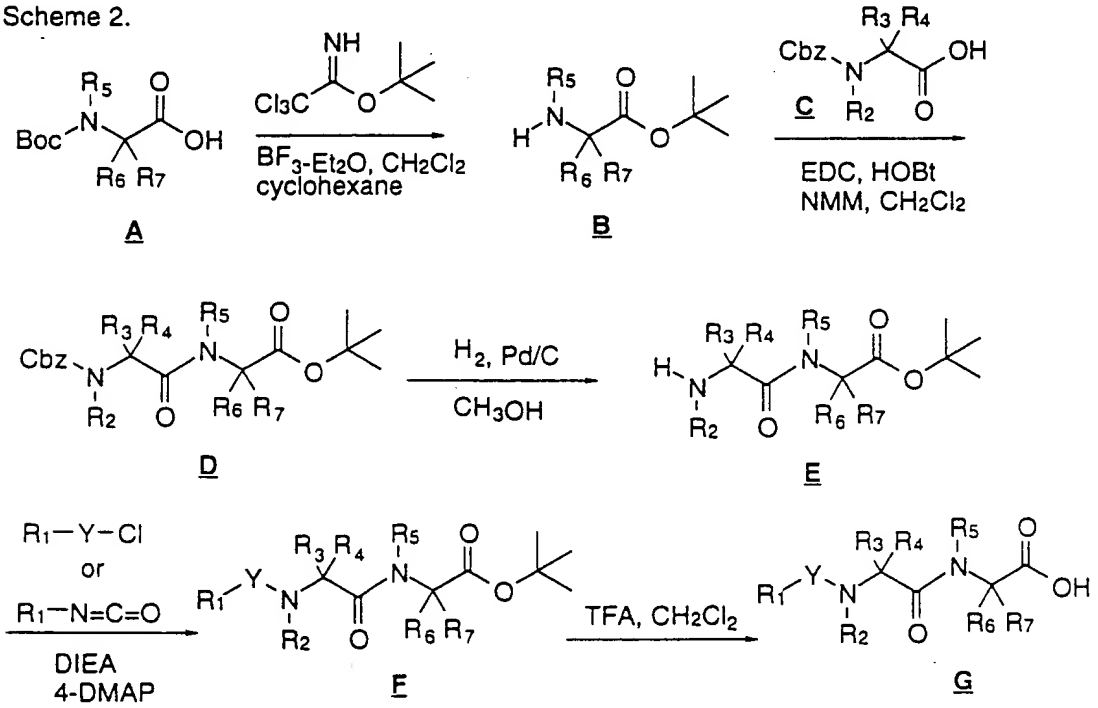
Scheme 1.



In the second method (Scheme 2), standard solution phase synthetic methodology is outlined. An N-Boc-protected amino acid derivative A (Boc = tert-butyloxycarbonyl) is treated with tert-butyl 2,2,2-trichloroacetimidate in the presence of boron trifluoride etherate to yield tert-butyl ester B which is subsequently coupled to Cbz-protected amino acid derivative C (Cbz = carbobenzyloxy) in the

presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), HOBt, and N-methylmorpholine (NMM) in methylene chloride (CH_2Cl_2) to yield dipeptide **D**. Catalytic hydrogenation of **D** in the presence of a palladium-on-carbon (Pd/C) catalyst yields **E**. Reaction of **E** with an acid chloride or isocyanate in the presence of DIEA and 4-dimethylaminopyridine (DMAP) yields **F** which is subsequently reacted with strong acid (TFA) to yield the desired product **G**.

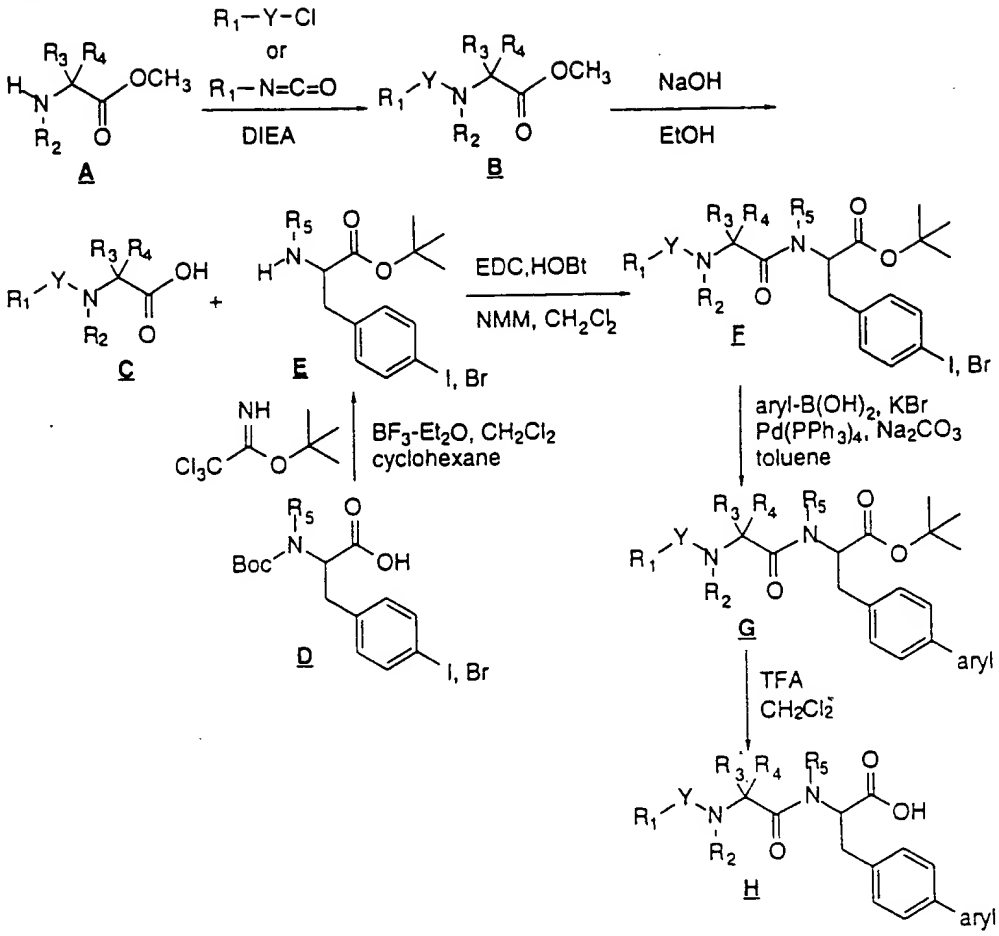
Scheme 2.



In the third method (Scheme 3), a late stage intermediate aryl bromide or iodide is coupled to an appropriately substituted aryl or heteroaryl boronic acid to give a subset of compounds of the present invention (R^6 = biaryl-substituted alkyl, R^7 = hydrogen). For example, amino acid methyl ester **A** is reacted with an acid chloride or isocyanate in the presence of DIEA to yield **B**. Basic hydrolysis of the methyl ester yields amino acid derivative **C**. N-Boc-4-iodo- or 4-bromo-phenylalanine **D** is reacted with *tert*-butyl 2,2,2-trichloroacetimidate in the presence of boron trifluoride etherate in methylene chloride-

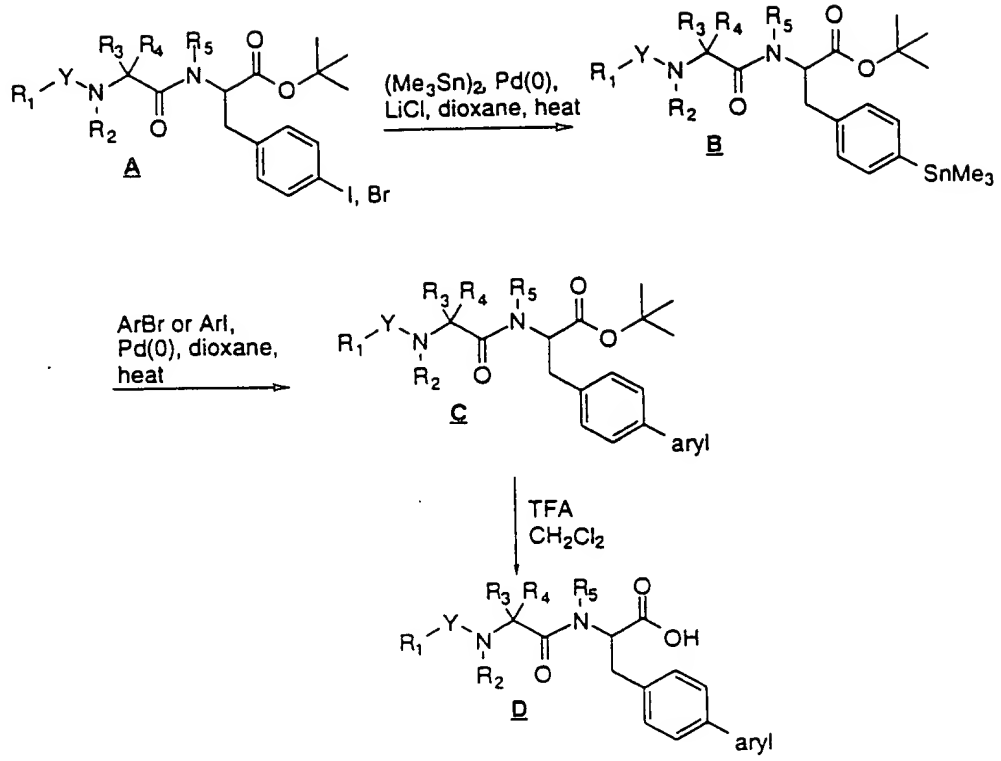
cyclohexane to yield tert-butyl ester **E** which is subsequently coupled with **C** in the presence of EDC, HOBt and NMM to yield 4-iodo- or 4-bromo-phenylalanine dipeptide **F**. Substituted aryl or heteroaryl boronic acids are coupled to **F** in the presence of a palladium(0) reagent, such as tetrakis(triphenylphosphine)palladium under Suzuki conditions (N. Miyaura *et al.*, *Synth. Commun.*, **1981**, *11*, 513-519) to yield **G**. The tert-butyl ester is then removed by treatment with strong acid (TFA) to yield the desired product **H**. If the aryl or heteroaryl boronic acid is not commercially available, but the corresponding bromide or iodide is, then the bromide or iodide can be converted into the desired boronic acid by treatment with an alkyllithium reagent in tetrahydrofuran at low temperature followed by addition of trimethyl or triisopropyl borate. Hydrolysis to the boronic acid can be effected by treatment of the intermediate with aqueous base and then acid.

Scheme 3.



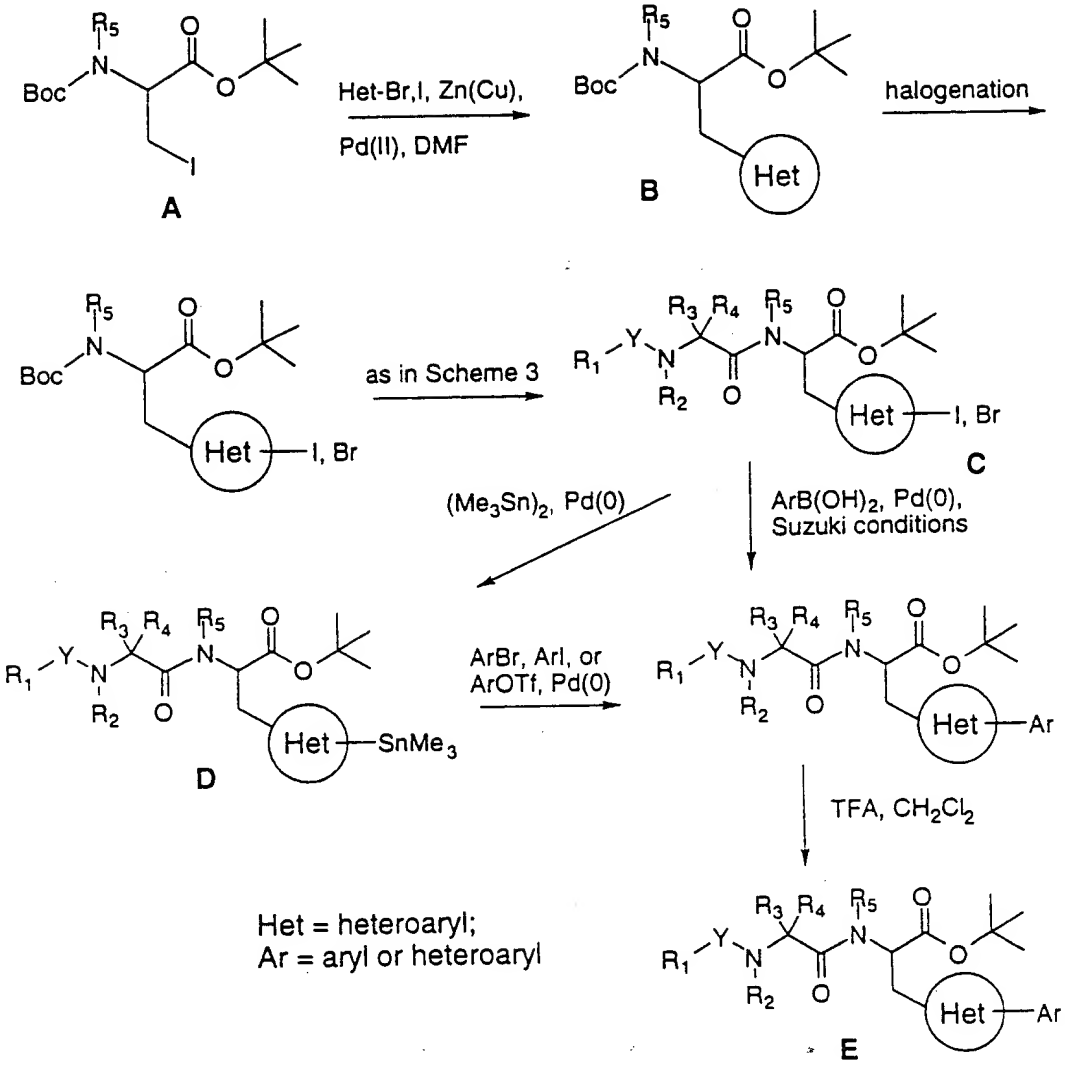
Alternatively, the aryl coupling reaction may be performed by application of Stille-type carbon-carbon bond forming conditions (Scheme 4). (A.M. Echavarren and J.K. Stille, *J. Am. Chem. Soc.* **1987**, *109*, 5478-5486). The aryl bromide or iodide intermediate **A** is converted into its trimethyltin derivative **B** using hexamethylditin in the presence of palladium(0) and lithium chloride and then reacted with an appropriately substituted aryl or heteroaryl bromide, iodide, or triflate in the presence of a palladium reagent, such as tetrakis(triphenylphosphine)palladium(0) or tris(dibenzylideneacetone)dipalladium(0), in a suitable solvent, such as toluene, dioxane, DMF, or 1-methyl-2-pyrrolidinone, to give intermediate **C**. The tert-butyl ester is then removed by treatment with strong acid (TFA) to yield the desired product **D**.

Scheme 4.



Compounds wherein the middle ring is heteroaryl (E) may be prepared (Scheme 5) in a similar fashion starting from the appropriate heteroaryl bromide or iodide C using Suzuki-type conditions as depicted in Scheme 3 or from the corresponding heteroaryl trimethyltin intermediate D using Stille-type conditions as depicted in Scheme 4. The requisite heteroaryl halides C may be prepared via conventional electrophilic halogenation of the N-Boc-heteroaryl-alanine *tert*-butyl ester intermediate B. B may be prepared from the known aliphatic iodo intermediate A in carbon-carbon bond formation using zinc/copper couple and palladium(II) (M.J. Dunn *et al.*, *SYNLETT* **1993**, 499-500).

Scheme 5.



General procedure for the solid-phase synthesis of compounds of Formula 1.

Step A. Loading of N-Fmoc-amino acid derivatives onto resins.

N-Fmoc-amino acids were loaded on either Wang® (Calbiochem-Novabiochem Corp.) or Chloro (2-chlorotrityl) resin. Wang® resin, typically 0.3 mmol, was washed with dimethylformamide three times. A solution of N-Fmoc-amino acid (0.3 mmol) in dimethylformamide (3 mL) was transferred to the pre-swollen Wang®

resin. Dicyclohexylcarbodiimide (0.3 mmol) and 1-N-hydroxybenztriazole (0.3 mmol) was added and the mixture gently swirled for 2 hours. Following filtration, the resin was sequentially washed with dimethylformamide (3 times) and dichloromethane (3 times). The amino acid substitution value obtained after vacuum drying typically ranged between 0.07 to 0.1 mmol.

Alternatively, Chloro (2-chorotrityl) resin, typically 0.2 mmol, was pre-swollen in dimethylformamide. A solution of N-Fmoc-amino acid (0.2 mmol) in dimethylformamide (3 ml) was added to the resin, followed by the addition of N,N-diisopropylethylamine(0.4 mmol). The resin was gently stirred for 2 hours, filtered and washed sequentially with dimethylformamide (3 times) and dichloromethane (3 times). The resin was finally washed with 10% methanol in dichloromethane and vacuum dried. The amino acid substitution value obtained after vacuum drying typically ranged between 0.05 to 0.1 mmol.

Step B. Deprotection of the N-Fmoc group.

The N-Fmoc protecting group was removed from the resin from Step A by treatment with 20% piperidine in dimethylformamide for 30 minutes. Following filtration, the resin was washed sequentially with dimethylformamide (3 times), dichloromethane (1 time) and dimethylformamide (2 times) and used in the subsequent reaction.

Step C. Coupling of the next N-Fmoc-amino acid derivative

A solution of the next desired N-Fmoc-amino acid derivative (0.4 mmol) in dimethylformamide (2 mL) was mixed with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.4 mmol), 1-hydroxybenzotriazole(0.4 mmol) and diisopropylethylamine (0.6 mmol). This solution was transferred to resin from Step B and typically allowed to react for 2 hours. Couplings were monitored by ninhydrin reaction. The coupling mixture was filtered and the resin washed with dimethylformamide (3 times) and used in the subsequent reaction.

Step D. Deprotection of the N-Fmoc group.

The N-Fmoc protecting group was removed from the resin from Step C by the procedure described in Step B and used in the subsequent reaction.

Step E. Acylation (or sulfonylation) of the terminal amino group.

The desired N-terminal capping reagent (sulfonyl chloride or other acyl chloride) (0.4 mol) was dissolved in dimethylformamide (2 ml), mixed with N,N-diisopropylethylamine(0.8 mmol) and added to the resin from Step D. After approximately two hours, the resin was sequentially washed with dimethylformamide (3 times) and dichloromethane (3 times).

Step F. Cleavage of the desired products from the resins.

The final desired products were cleaved from the resins from Step E by gently stirring with a solution of trifluoroacetic acid:thioanisole:ethanedithiol (95:2.5:2.5); 3 hours for Wang® resin and 30 minutes for the Chloro (2-chorotrityl) resin. Following filtration, the solvents were removed by evaporation and the residue dissolved in acetonitrile (3 mL). Insoluble material was removed by filtration. The final products were purified by reverse phase chromatography with a linear gradient of buffer A (0.1% trifluoroacetic acid in water) and buffer B (0.1% trifluoroacetic acid in acetonitrile) and isolated by lyophilization. Molecular ions were obtained by electrospray ionization mass spectrometry or matrix-assisted laser desorption ionization time-of-flight mass spectrometry to confirm the structure of each peptide.

The following compounds were prepared by the above general procedures using the appropriate amino acid derivatives and acylor sulfonyl chloride:

Example	Compound Name	MS *

(1)	N-(3,4-dimethoxybenzenesulfonyl)-1,2,3,4-tetrahydro-isoquinoline-3(S)-carbonyl-(L)-biphenylalanine	601
(2)	N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-biphenylalanine	548

* m/e, M+1

EXAMPLE 3

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(4-fluorophenyl)phenylalanine

Step A: 4-Iodo-(L)-Phenylalanine, tert-butyl ester hydrochloride.

To a suspension of N-Boc-4-iodo-(L)-phenylalanine (1.0 g, 2.56 mmol) in methylene chloride (7 mL) and cyclohexane (14 mL) were added t-butyl trichloroacetimidate (0.48 mL, 2.68 mmol) and boron trifluoride-etherate (48 μL). The reaction mixture was stirred for 5 hours at room temperature under a nitrogen atmosphere and then treated a second time with the same amounts of t-butyl trichloroacetimidate and boron trifluoride-etherate as above. After stirring overnight, a third addition was made, and the mixture was stirred a further 3 hours. The mixture was then filtered and the filtrate evaporated. The product was obtained pure by silica gel chromatography eluting with 10% diethyl ether in hexane; yield 650 mg. The product was treated with 1M HCl in ethyl acetate (7.3 mL) for 18 hours at room temperature. The mixture was evaporated and coevaporated several times with diethyl ether to afford the title compound; yield 522 mg.
400 MHz ^{1}H NMR (CD_3OD): δ 1.42 (s, 9H); 3.13 (d, 2H); 4.18 (t, 1H); 7.09 (d, 2H); 7.75 (d, 2H).

Step B: N-(3,5-Dichlorobenzenesulfonyl)-(L)-proline

To a mixture of (L)-proline methyl ester hydrochloride (838 mg, 5.06 mmol) in methylene chloride (25 mL) at 0°C were added N,N-diisopropylethylamine (2.64 mL, 15.2 mmol) and a solution of 3,5-

dichlorobenzenesulfonyl chloride (1.49 g, 6.07 mmol) in methylene chloride (5 mL). The cooling bath was removed, and the mixture was stirred overnight at room temperature. It was then diluted with methylene chloride, washed with 1N hydrochloric acid, saturated $NaHCO_3$, saturated brine solution, dried (Na_2SO_4), and evaporated. The methyl ester was obtained pure by silica gel chromatography eluting with 10% acetone in hexane; yield 1.49 g. It was then taken up in ethanol (50 mL) and treated with 0.2 N sodium hydroxide (26.6 mL) for 1.5 hours at room temperature. The mixture was acidified with glacial acetic acid, concentrated, the residue taken up in methylene chloride, washed with water, saturated brine solution, dried (Na_2SO_4), and evaporated to give the title compound; yield 1.4 g.
400 MHz ^1H NMR (CD_3OD): δ 1.80-2.15 (m, 4H); 3.35-4.45 (m, 2H); 4.30 (dd, 1H); 7.76 (m, 1H); 7.83 (m, 2H).

Step C: N-(3,5-Dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-iodophenylalanine, tert-butyl ester.

To a solution of N-(3,5-dichlorobenzenesulfonyl)-(L)-proline (386 mg, 1.19 mmol) in methylene chloride (23 mL) were added 1-hydroxybenzotriazole (241 mg, 1.79 mmol), N-methylmorpholine (0.33 mL, 2.98 mmol), and 4-iodo-(L)-phenylalanine tert-butyl ester hydrochloride (458 mg, 1.19 mmol). After cooling in an ice-bath for 5 minutes, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (274 mg, 1.43 mmol) was added. After 15 minutes, the cooling bath was removed, and the mixture was stirred overnight under a nitrogen atmosphere. The mixture was diluted with methylene chloride, washed with water, 1N HCl, saturated $NaHCO_3$ solution, saturated brine solution, dried ($MgSO_4$), and evaporated. Silica gel chromatography eluting with 20% ethyl acetate in hexane afforded pure title compound; yield 651 mg (84%).
MS: m/e 653 (M + 1)
400 MHz ^1H NMR (CD_3OD): δ 1.45 (s, 9H); 1.65-1.85 (m, 4H); 3.0 (dd, 1H); 3.13 (dd, 1H); 3.45 (m, 1H); 4.20 (m, 1H); 4.55 (dd, 1H); 7.05 (d, 2H); 7.64 (d, 2H); 7.80 (s, 3H).

Step D: N-(3,5-Dichlorobenzenesulfonyl)-(L)-Prolyl-(L)-4-(4-fluorophenyl)phenylalanine, tert-butyl ester.

To a solution of N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-4-iodo-(L)-phenylalanine tert-butyl ester (100 mg, 0.15 mmol) in toluene (1 mL) and ethanol (0.5 mL) were added 4-fluorobenzeneboronic acid (24 mg, 0.16 mmol), potassium bromide (20 mg, 0.17 mmol), 2M Na_2CO_3 (0.20 mL, 0.38 mmol), and tetrakis(triphenylphosphine)palladium (9 mg, 0.008 mmol). The mixture was stirred for 1.5 hours at 95°C under a nitrogen atmosphere, allowed to cool to room temperature, diluted with ethyl acetate, washed twice with 1N sodium hydroxide, once with saturated brine solution, dried ($MgSO_4$), and evaporated. The title compound was obtained pure by silica gel chromatography eluting with 10% acetone in hexane; yield 36 mg (38%).

MS: m/e 621 (M + H); 638 (M + H + NH_3)

400 MHz ^1H NMR (CD_3OD): δ 1.47 (s, 9H); 1.65-1.87 (m, 4H); 3.08 (dd, 1H); 3.20 (dd, 1H); 3.45 (m, 1H); 4.24 (dd, 1H); 4.63 (dd, 1H); 7.15 (t, 2H); 7.35 (d, 2H); 7.54 (d, 2H); 7.57 (m, 2H); 7.77-7.80 (m, 3H).

Step E: N-(3,5-Dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(4'-fluorophenyl)phenylalanine.

A cooled solution of N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-4-(4-fluorophenyl)-(L)-phenylalanine tert-butyl ester (36 mg, 0.055 mmol) in methylene chloride (1.4 mL) was treated with trifluoroacetic acid (0.28 mL, 3.63 mmol). The cooling bath was removed, and the mixture was stirred overnight at room temperature. The reaction mixture was then evaporated, coevaporated with methylene chloride (3X), toluene (2X), and finally methanol. The product was dried under high vacuum; yield 32 mg.

MS: m/e 565 (M + H); 582 (M + H + NH_3)

400 MHz ^{1}H NMR (CD_3OD): δ 1.60-1.90 (m, 4H); 3.10 (dd, 1H); 3.42 (m, 1H); 4.22 (t, 1H); 4.73 (m, 1H); 7.11 (t, 2H); 7.34 (d, 2H); 7.52 (d, 2H); 7.56 (m, 2H); 7.72-7.79 (m, 3H).

EXAMPLE 4

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(2'-thienyl)-phenylalanine

This compound was prepared in a similar fashion as Example 3 using 2-thienyl-boronic acid in the Suzuki coupling reaction; MS: m/e 553 (M + 1); 570 (M + 1 + NH_3).

EXAMPLE 5

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(3'-thienyl)-phenylalanine

This compound was prepared in a similar fashion as Example 3 using 3-thienyl-boronic acid in the Suzuki coupling reaction; MS: m/e 553 (M + 1); 570 (M + 1 + NH_3).

EXAMPLE 6

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(4'-trifluoromethyl-phenyl)-phenylalanine

This compound was prepared in a similar fashion as Example 3 using 4-trifluoromethylbenzene boronic acid in the Suzuki coupling reaction; MS: m/e 615 (M + 1); 632 (M + 1 + NH_3).

EXAMPLE 7

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(2'-methoxy-phenyl)-phenylalanine

This compound was prepared in a similar fashion as Example 3 using 2-methoxy-benzene boronic acid in the Suzuki coupling reaction; MS: m/e 577 (M + 1); 594 (M + 1 + NH_3).

EXAMPLE 8

N-(3,5-Dichlorobenzenesulfonyl)-(L-)-prolyl-(L)-4-(2'-formyl-phenyl)-phenylalanine

This compound was prepared in a similar fashion as Example 3 using 2-formyl-benzene boronic acid in the Suzuki coupling reaction; MS: m/e 575 (M + 1); 592 (M + 1 + NH_3).

EXAMPLE 9

Inhibition of VLA-4 Dependent Adhesion to BSA-CS-1 Conjugate

Step 1. Preparation of CS-1 Coated Plates

Untreated 96 well polystyrene flat bottom plates were coated with bovine serum albumin (BSA; 20 µg/ml) for 2 hours at room temperature and washed twice with phosphate buffered saline (PBS). The albumin coating was next derivatized with 10 µg/ml 3-(2-pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP), a heterobifunctional crosslinker, for 30 minutes at room temperature and washed twice with PBS. The CS-1 peptide (Cys-Leu-His-Gly-Pro-Glu-Ile-Leu-Asp-Val-Pro-Ser-Thr), which was synthesized by conventional solid phase chemistry and purified by reverse phase HPLC, was next added to the derivatized BSA at a concentration of 2.5 µg/ml and allowed to react for 2 hours at room temperature. The plates were washed twice with PBS and stored at 4°C.

Step 2. Preparation of Fluorescently Labeled Jurkat Cells

Jurkat cells, clone E6-1, obtained from the American Type Culture Collection (Rockville, MD; cat # ATCC TIB-152) were grown and maintained in RPMI-1640 culture medium containing 10% fetal calf serum (FCS), 50 units/ml penicillin, 50 μg/ml streptomycin and 2 mM L-Glutamine. Fluorescence activated cell sorter analysis with specific monoclonal antibodies confirmed that the cells expressed both the α4 and β1 chains of VLA-4. The cells were centrifuged at 400xg for five minutes and washed twice with PBS. The cells were incubated at a concentration of 2 x 10^6 cells/ml in PBS containing a 1 μM concentration of a fluorogenic esterase substrate (2', 7'-bis-(2-carboxyethyl)-5-(and -6)-carboxyfluorescein, acetoxymethyl ester; BCECF-AM; Molecular Probes Inc., Eugene, Oregon; catalog #B-1150) for 30-60 minutes at 37°C in a 5% CO_2/air incubator. The fluorescently labeled Jurkat cells were washed two times in PBS and resuspended in RPMI containing 0.25% BSA at a final concentration of 2.0 x 10^6 cells/ml.

Step 3. Assay Procedure

Compounds of this invention were prepared in DMSO at 100x the desired final assay concentration. Final concentrations were selected from a range between 0.001 nM-100μM. Three μL of diluted compound, or vehicle alone, were premixed with 300 μL of cell suspension in 96-well polystyrene plates with round bottom wells. 100 μL aliquots of the cell /compound mixture were then transferred in duplicate to CS-1 coated wells. The cells were next incubated for 30 minutes at room temperature. The non-adherent cells were removed by two gentle washings with PBS. The remaining adherent cells were quantitated by reading the plates on a Cytofluor II fluorescence plate reader (Perseptive Biosystems Inc., Framingham, MA; excitation and emission filter settings were 485 nm and 530 nm, respectively). Control

wells containing vehicle alone were used to determine the level of cell adhesion corresponding to 0% inhibition. Wells in which cells were treated with a saturating concentration (10 ng/ml) of a neutralizing anti-α4 antibody (HP 2/1; Immunotech, Inc., Westbrook, ME) were used to determine the level of cell adhesion corresponding to 100% inhibition. Cell adhesion in the presence of HP2/1 was usually less than 5% of that observed in the presence of vehicle alone. Percent inhibition was then calculated for each test well and the IC_{50} was determined from an eight point titration using a validated four parameter fit algorithm.

EXAMPLE 10

Antagonism of VLA-4 Dependent Binding to VCAM-Ig Fusion Protein.

1. Preparation of VCAM-Ig

The signal peptide as well as domains 1 and 2 of human VCAM (GenBank Accession no. M30257) were amplified by PCR using the human VCAM cDNA (R & D Systems) as template and the following primer sequences: 3'-PCR primer:5'-
AATTATAATTTGATCAACTTAC
CTGTCAATTCTTTTACAGCCTGCC-3';
5'-PCR primer:
5'-ATAGGAATTCCAGCTGCCACCATGCCTGGGAAGATGGTCG-3'. The 5'-PCR primer contained EcoRI and PvuII restriction sites followed by a Kozak consensus sequence (CCACC) proximal to the initiator methionine ATG. The 3'-PCR primer contained a BclI site and a splice donor sequence. PCR was performed for 30 cycles using the following parameters: 1 min. at 94°C, 2 min. at 55°C and 2 min. at 72°C. The amplified region encoded the following sequence of human VCAM:
MPGKMVVILGASNILWIMFAASQAFKIETTPESRYLAQIGDSVSLT
CSTTGCESPFFSWRTQIDSPLNGKVTNEGTTSTLTMNPVSFGNEHS
YLCTATCESRKLEKGIQVEIYSFPKDPEIHLSGPLEAGKPITVKCSV

ADVYPFDRLEIDLLKGDHLMKSQEFLEDADRKSLETKSLEVTFTP VIEDIGKVLVCRAKLHIDEMDSVPTVRQAVKEL. The resulting PCR product of 650 bp was digested with EcoRI and BclI and ligated to expression vector pIg-Tail (R & D Systems, Minneapolis, MN) digested with EcoRI and BamHI. The pIg-Tail vector contains the genomic fragment which encodes the hinge region, CH2 and CH3 of human IgG1 (GenBank Accession no. Z17370). The DNA sequence of the resulting VCAM fragment was verified using Sequenase (US Biochemical, Cleveland, OH). The fragment encoding the entire VCAM-Ig fusion was subsequently excised from pIg-Tail with EcoRI and NotI and ligated to pCI-neo (Promega, Madison, WI) digested with EcoRI and NotI. The resulting vector, designated pCI-neo/VCAM-Ig was transfected into CHO-K1 (ATCC CCL 61) cells using calcium-phosphate DNA precipitation (Specialty Media, Lavalette, NJ). Stable VCAM-Ig producing clones were selected according to standard protocols using 0.2-0.8 mg/ml active G418 (Gibco, Grand Island, NY), expanded, and cell supernatants were screened for their ability to mediate Jurkat adhesion to wells previously coated with 1.5 µg/ml (total protein) goat anti-human IgG (Sigma, St. Louis, MO). A positive CHO-K1/VCAM-Ig clone was subsequently adapted to CHO-SFM serum-free media (Gibco) and maintained under selection for stable expression of VCAM-Ig. VCAM-Ig was purified from crude culture supernatants by affinity chromatography on Protein A/G Sepharose (Pierce, Rockford, IL) according to the manufacturer's instructions and desalted into 50 mM sodium phosphate buffer, pH 7.6, by ultrafiltration on a YM-30 membranes (Amicon, Beverly, MA).

Step 2. Preparation of ^{125}I-VCAM-Ig

VCAM-Ig was labeled to a specific radioactivity greater that 1000 Ci/mmole with ^{125}I-Bolton Hunter reagent (New England Nuclear, Boston, MA; cat # NEX120-0142) according to the manufacturer's instructions. The labeled protein was separated from unincorporated isotope by means of a calibrated HPLC gel filtration

column (G2000SW; 7.5 x 600 mm; Tosoh, Ja n) using uv and radiometric detection.

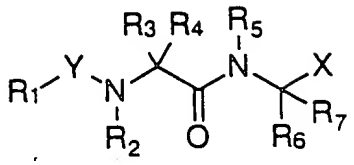
Step 3. VCAM-Ig Binding Assay

Compounds of this invention were prepared in DMSO at 100x the desired final assay concentration. Final concentrations were selected from a range between 0.001 nM-100μM. Jurkat cells were centrifuged at 400xg for five minutes and resuspended in binding buffer (25 mM HEPES, 150 mM NaCl, 3 mM KCl, 2 mM glucose, 1 mM $MnCl_2$, 0.1% bovine serum albumin, pH 7.4) without $MnCl_2$. The cells were centrifuged again and resuspended in complete binding buffer. Compounds were assayed in Millipore MHVB multiscreen plates (cat# MHVBN4550, Millipore Corp., MA) by making the following additions to duplicate wells: (i) 200 μL of binding buffer; (ii) 20 μL of a working stock of ^{125}I-VCAM-Ig prepared in binding buffer (final assay concentration ≤ 100 pM); (iii) 2.5 μL of compound solution or vehicle alone; (iv) and 0.5 x 10^6 cells in a volume of 30 μL. The plates were incubated at room temperature for 30 minutes, filtered on a vacuum box, and washed on the same apparatus by the addition of 100 μL of binding buffer. After insertion of the multiscreen plates into adapter plates (Packard, Meriden, CT, cat# 6005178), 100 μL of microscint-20 (Packard cat# 6013621) was added to each well. The plates were then sealed, placed on a shaker for 30 seconds, and counted on a Topcount microplate scintillation counter (Packard). Control wells containing vehicle alone were used to determine the level of VCAM-Ig binding corresponding to 0% inhibition. Wells in which cells were treated with a saturating concentration of unlabeled VCAM-Ig (10 nM) were used to determine the level of binding corresponding to 100% inhibition. Binding of ^{125}I-VCAM-Ig in the presence of 10 nM unlabeled VCAM-Ig was usually less than 5% of that observed in the presence of vehicle alone. Percent inhibition was then calculated for each test well and the IC_{50} was determined from a ten point titration using a validated four parameter fit algorithm. Compounds of Examples 1-8 showed IC50

values of less than 10 nM in the inhibition of VCAM-Ig binding to VLA-4.

WHAT IS CLAIMED IS:

1. A compound of Formula I



I

or a pharmaceutically acceptable salt thereof wherein:

R^1 is 1) C_{1-10}alkyl,
2) C_{2-10}alkenyl,
3) C_{2-10}alkynyl,
4) Cy,
5) Cy-C_{1-10}alkyl,
6) Cy-C_{2-10}alkenyl,
7) Cy-C_{2-10}alkynyl,

wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a; and Cy is optionally substituted with one to four substituents independently selected from R^b;

R^2 and R^3 are independently
1) hydrogen, or
2) a group selected from R^1; or

R^2 and R^3 together with the atoms to which they are attached form a ring of 4 to 7 members containing 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein said ring may be isolated or benzo-fused, and optionally substituted with one to four substituents independently selected from R^b;

R^4 and R^7 are independently selected from the group consisting of
1) hydrogen,
2) C_{1-10}alkyl,

3) C_{2-10}alkenyl,
4) C_{2-10}alkynyl,
5) aryl,
6) aryl C_{1-10}alkyl,
7) heteroaryl, and
8) heteroaryl C_{1-10}alkyl,

wherein alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents independently selected from R^a, and aryl and heteroaryl are optionally substituted with one to four substituents independently selected from R^b; or

R3, R4 and the carbon to which they are attached form a 3-7 membered ring optionally containing 0-2 heteroatoms selected from N, O and S;

R^5 is 1) hydrogen,
2) C_{1-10}alkyl optionally substituted with one to four substituents independently selected from R^a, or
3) Cy optionally substituted with one to four substituents independently selected from R^b,

R^6 is 1) Ar^1-Ar^2-C_{1-10}alkyl,
2) Ar^1-Ar^2-C_{2-10}alkenyl,
3) Ar^1-Ar^2-C_{2-10}alkynyl,

wherein Ar^1 and Ar^2 are independently aryl or heteroaryl each of which is optionally substituted with one to four substituents independently selected from R^b; alkyl, alkenyl and alkynyl are optionally substituted with one to four substituents independently selected from R^a;

R^a is 1) Cy
2) $-OR^d$,
3) $-NO_2$,
4) halogen
5) $-S(O)_mR^d$,

6) $-SR^d$,
7) $-S(O)_2OR^d$,
8) $-S(O)_mNR^dR^e$,
9) $-NR^dR^e$,
10) $-O(CR^fR^g)_nNR^dR^e$,
11) $-C(O)R^d$,
12) $-CO_2R^d$,
13) $-CO_2(CR^fR^g)_nCONR^dR^e$,
14) $-OC(O)R^d$,
15) -CN,
16) $-C(O)NR^dR^e$,
17) $-NR^dC(O)R^e$,
18) $-OC(O)NR^dR^e$,
19) $-NR^dC(O)OR^e$,
20) $-NR^dC(O)NR^dR^e$,
21) $-CR^d(N-OR^e)$, or
22) $-CF_3$;

wherein Cy is optionally subsituted with one to four substituents independently selected from R^c;

R^b is 1) a group selected from R^a,
2) C_{1-10} alkyl,
3) C_{2-10} alkenyl,
4) C_{2-10} alkynyl,
5) aryl C_{1-10}alkyl,
6) heteroaryl C_{1-10} alkyl,

wherein alkyl, alkenyl, alkynyl, aryl, heteroaryl are optionally substituted with a group independently selected from R^c;

R^c is 1) halogen,
2) amino,
3) carboxy,
4) C_{1-4}alkyl,
5) C_{1-4}alkoxy,

6) aryl,
7) aryl C_{1-4}alkyl, or
8) aryloxy.

R^d and R^e are independently selected from hydrogen, C_{1-10}alkyl, C_{2-10}alkenyl, C_{2-10}alkynyl, Cy and Cy C_{1-10}alkyl, wherein alkyl, alkenyl, alkynyl and Cy is optionally substituted with one to four substituents independently selected from R^c; or
R^d and R^e together with the atoms to which they are attached form a heterocyclic ring of 5 to 7 members 0-2 additional heteroatoms independently selected from oxygen, sulfur and nitrogen;

R^f and R^g are independently selected from hydrogen, C_{1-10}alkyl, Cy and Cy C_{1-10}alkyl; or
R^f and R^g together with the carbon to which they are attached form a ring of 5 to 7 members containing 0-2 heteroatoms independently selected from oxygen, sulfur and nitrogen;

R^h is 1) hydrogen,
2) C_{1-10}alkyl,
3) C_{2-10}alkenyl,
4) C_{2-10}alkynyl,
5) cyano,
6) aryl,
7) aryl C_{1-10}alkyl,
8) heteroaryl,
9) heteroaryl C_{1-10}alkyl, or
10) $-SO_2R^i$;
wherein alkyl, alkenyl, and alkynyl are optionally substituted with one to four substituents independently selected from R^a; and aryl and heteroaryl are each optionally substituted with one to four substituents independently selected from R^b;

R^i 1) C_{1-10}alkyl,

2) C_{2-10}alkenyl,
3) C_{2-10}alkynyl, or
4) aryl;

wherein alkyl, alkenyl, alkynyl and aryl are each optionally substituted with one to four substituents independently selected from R^c;

Cy is cycloalkyl, heterocyclyl, aryl, or heteroaryl;

m is an integer from 1 to 2;

n is an integer from 1 to 10;

X is 1) $-C(O)OR^d$,
2) $-P(O)(OR^d)(OR^e)$
3) $-P(O)(R^d)(OR^e)$
4) $-S(O)_mOR^d$,
5) $-C(O)NR^dR^h$, or
6) -5-tetrazolyl;

Y is 1) -C(O)-,
2) -O-C(O)-,
3) $-NR^e$-C(O)-,
4) $-S(O)_2$-,
5) $-P(O)(OR^i)$
6) C(O)C(O).

2. A compound of Claim 1 selected from the group consisting of:
N-(3,4-dimethoxybenzenesulfonyl)-1,2,3,4-tetrahydroisoquinoline-3(S)-carbonyl-(L)-4-biphenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-biphenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L-prolyl-(L)-4-(4-fluorophenyl)phenylalanine;

N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-thienyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(3'-thienyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(4'-trifluoromethyl-phenyl)-phenylalanine;
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-methoxy-phenyl)-phenylalanine; and
N-(3,5-dichlorobenzenesulfonyl)-(L)-prolyl-(L)-4-(2'-formyl-phenyl)-phenylalanine.

3. A method for inhibiting cell adhesion in a mammal which comprises administering to said mammal an effective amount of a compound of Claim 1.

4. A method for the treatment of diseases, disorders, conditions or symptoms mediated by cell adhesion in a mammal which comprises administering to said mammal an effective amount of a compound of Claim 1.

5. A method for the treatment of asthma, allergic rhinitis, multiple sclerosis, atherosclerosis or inflammation in a mammal which comprises administering to said mammal an effective amount of a compound of Claim 1.

6. A pharmaceutical composition which comprises a compound of Claim 1 and a pharmaceutically acceptable carrier thereof.

7. A method for inhibiting cell adhesion in a mammal which comprises administering to said mammal an effective amount of a compound of Claim 2.

8. A method for the treatment of diseases, disorders, conditions or symptoms mediated by cell adhesion in a mammal which

comprises administering to said mammal an effective amount of a compound of Claim 2

9. A method for the treatment of asthma, allergic rhinitis, multiple sclerosis, atherosclerosis or inflammation in a mammal which comprises administering to said mammal an effective amount of a compound of Claim 2.

10. A pharmaceutical composition which comprises a compound of Claim 2 and a pharmaceutically acceptable carrier thereof.

TITLE OF THE INVENTION

BIARYLALKANOIC ACID COMPOUNDS S AS CELL ADHESION INHIBITORS

ABSTRACT OF THE DISCLOSURE

Biarylalkanoic acids of Formula I are antagonists of VLA-4, and as such are useful in the inhibition of prevention of cell adhision and cell-adhesion mediated pathologies. These compounds may be formulated into pharmaceutical compositions and are suitable for use in the treatment of asthma, allergies, inflammation, multiple sclerosis, and other inflammatory and autoimmune disorders.